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Abundant Production of Brain-Derived Neurotrophic Factor by Adult Visceral Epithelia

Implications for Paracrine and Target-Derived Neurotrophic Functions

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Brain-derived neurotrophic factor (BDNF) plays a crucial role for the survival of visceral sensory neurons during development. However, the physiological sources and the function of BDNF in the adult viscera are poorly described. We have investigated the cellular sources and the potential role of BDNF in adult murine viscera. We found markedly different amounts of BDNF protein in different organs. Surprisingly, BDNF levels in the urinary bladder, lung, and colon were higher than those found in the brain or skin. *In situ* hybridization experiments revealed that BDNF mRNA was made by visceral epithelial cells, several types of smooth muscle, and neurons of the myenteric plexus. Epithelia that expressed BDNF lacked both the high- and low-affinity receptors for BDNF, trkB and $p75^{NTR}$. In contrast, both receptors were present on neurons of the peripheral nervous system. Studies with BDNF−/−mice demonstrated that epithelial and smooth muscle cells developed normally in the absence of BDNF. These data provide evidence that visceral epithelia are a major source, but not a target, of BDNF in the adult viscera. The abundance of BDNF protein in certain internal organs suggests that this neurotrophin may regulate the function of adult visceral sensory and motor neurons. ***(Am J Pathol 1999, 155:1183–1193)***

Brain-derived neurotrophic factor (BDNF) supports the survival, differentiation, and function of a broad number of central nervous system (CNS) and peripheral nervous system (PNS) neurons.[1] The tyrosine kinase trkB was identified as the high-affinity receptor and $p75^{NTR}$ as the low-affinity receptor for BDNF.[2,3] Initially, BDNF expression was thought to be restricted to the CNS.[4] Barde and colleagues, however, showed that sub-populations of sensory neurons are BDNF responsive during development.[5–7] Studies with BDNF knockout (−/−) mice definitively demonstrated a crucial role of BDNF for the survival of developing PNS neurons. BDNF−/− mice display an extensive loss of visceroafferent neurons in the nodose (70%), trigeminal (40%), and dorsal root ganglia (30%).[8–11] These mice develop sensory deficits, severe respiratory problems, and abnormalities in feeding and behavior and die within 3 weeks after birth.[9,12]

Though there is good evidence for the fundamental role of target-derived BDNF for the development of visceral innervation,[13,14] the role of target-derived BDNF for adult visceral neurons is rather unknown. Recently, it has been observed that inflammatory diseases of the adult viscera are associated with a strong increase in local BDNF mRNA and protein production.[15–17] These observations raised the possibility that BDNF might mediate changes in neuronal function in pathological conditions, in that there is growing evidence for a functional role for BDNF in the normal adult peripheral nervous system.[18–21] The involvement of target-derived mechanisms has been suggested, because there is recent evidence for retrograde transport of BDNF in adult visceroafferent and visceroefferent neurons.[22] This is supported by the finding that there are many more neurons in the adult nodose and petrosal ganglion (NPG) and (DRG) that contain BDNF protein than produce BDNF mRNA.[23,24] Though target-derived actions of BDNF in the adult viscera have been discussed, a systematic study of BDNF expression in the viscera is still lacking. Moreover, most

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reports do not identify the cellular sources of BDNF. There is some evidence for the presence of BDNF mRNA in extracts from the lung, heart, and spleen[25–27] and of BDNF protein in extracts of the rat liver and thymus.[28] As possible physiological sources of BDNF, only fibroblasts,[29–31] vascular smooth muscle cells,[32,33] and thymic stroma cells have been identified so far.[34]

It was the aim of this study, therefore, to investigate systematically the expression and potential role of BDNF in the targets of adult visceral sensory and motor neurons. Using a nonradioactive *in situ* hybridization technique, which gives very good cellular resolution, we identified the cells synthesizing BDNF mRNA in all gastrointestinal regions and in tissues of the cardiorespiratory and urogenital systems. In addition, we quantified the amounts of BDNF protein present in these internal organs. Furthermore, we have examined the distribution of BDNF receptors and the morphology of viscera in mice lacking BDNF. We found that BDNF is expressed in certain viscera in even higher amounts than in the brain. The distribution of BDNF receptors and the phenotype of BDNF−/− mice suggest a primarily neurotrophic role for BDNF made by visceral epithelia. We conclude that visceral BDNF could indeed regulate functional properties of adult PNS neurons.

Materials and Methods

Animals

Female Balb/c mice were obtained from Harlan-Winkelmann (Borchen, Germany). FVB/N transgenics were genotyped by polymerase chain reaction (PCR) analysis as described before.[35,36] Wild-type and BDNF−/− mice were obtained from the mating of BDNF+/− mice maintained at the Max Delbrück Centrum, Berlin. The production and maintenance of these mice have been described elsewhere.[21] Paraffin sections (2 μm) of internal organs from 2-week-old wild-type (+/+) and BDNF−/− mice were stained with hematoxylin-eosin (HE) following standard laboratory procedures.

In Situ *Hybridization (ISH)*

The riboprobe for BDNF was prepared as described by Schaeren-Wiemers and Gerfin-Moser.[37] Briefly, for *in vitro* transcription 1 μg of linearized plasmid containing 510 bp of the BDNF coding sequence (nucleotides 224–734) was used as a template.[38] The reaction was performed in a 50-μl volume using the DIG-RNA-labeling mix from Boehringer Mannheim (Mannheim, Germany) and a T7 (anti-sense) or T3 (sense control) polymerase (Promega, Madison, WI). After a 3-hour incubation the reaction was stopped by adding DNase. The probe was hydrolyzed by adding two volumes of carbonate buffer (60 mmol/L Na_2CO_3, 40 mmol/L $NaHCO_3$, pH 10.2) followed by 45 minutes' incubation at 60°C. After neutralization with an equal volume of neutralization buffer (200 mmol/L Na-acetate, 1% acetic acid, pH 6.0), the probe was purified by ethanol precipitation. To estimate the concentration of the probe a dot blot was performed as recommended by the manufacturer (Boehringer Mannheim) for nonradioactive ISH. Probes were stored at −80°C.

Organs from 8-week-old Balb/c mice were immediately frozen in Tissue Tek (Miles, Elkhart, IN). Slides were coated with 2% APES (3-Aminopropyltriethoxysilane, Sigma, Deisenhofen, Germany) in Aceton under RNase-free conditions. Ten-micron cryosections were dried for 30 minutes, fixed in 4% cold paraformaldehyde for 10 minutes, and then washed in RNase-free PBS. Two sequential sections of each organ were used for anti-sense and sense staining. After acetylation, sections were washed in PBS. For prehybridization, 500 μl of hybridization buffer (50% formamide, 4× SSC, 2× Denhardt's solution, 50 μg/ml RNA-core from baker's yeast) were added to each slide. The slides were placed in a humid chamber containing a 50% formamide/4× SSC mix at the bottom. The hybridization mixture was prepared by adding 150 ng/ml digoxigenin (DIG)-labeled cRNA (anti-sense or sense) to the hybridization buffer. To denature the probe the mixture was incubated at 85°C for 5 minutes. The prehybridization solution was allowed to drip off the slides, and 200 μl of hybridization mixture were added to each slide. Hybridization was performed overnight at 56°C. Posthybridization washes were carried out in the following sequence: 4× 10 minutes in 2× SSC at 67°C, 45 minutes in 2× SSC at 67°C, 60 minutes in 0.1× SSC at 67°C, 10 minutes in 0.2× SSC at room temperature. Detection of the DIG-labeled probe was performed as described in the manufacturer's instructions, with antibody incubation overnight at 4°C. Color development was allowed to proceed in the dark for 2 hours. The reaction was terminated by immersing the slides in PBS, pH 7.5.

Preparation of Tissue Lysates

Organs from 8-week-old Balb/c mice were prepared and pulverized in liquid nitrogen. The lysing buffer contained 50 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonylfluoride, 5 mmol/L Iodacetamid, 10 mg/ml aprotinin, and (as detergents) 0.2% SDS, 1% Triton X-100, and 1% Igepal (Sigma). Protein extraction was performed as described before.[39] The lysing solutions were centrifuged at 2000 × *g*, 4°C, for 30 minutes and supernatants stored at −80°C. Total protein content was measured using the detergent-compatible BCA protein assay (Pierce, Rockford, IL).

Determination of BDNF Protein by Enzyme-Linked Immunosorbent Assay (ELISA)

BDNF in lysates was measured using commercial ELISA kits according to the manufacturer's instructions (Promega) as described.[17] The detection limit was 4 pg/ml. Measurements were performed in duplicate. Concentrations of BDNF were calculated as nanograms of BDNF per gram of total protein.

trkB and p75^{NTR} Immunoreactivity

Immunoreactivity against the full-length form of mouse trkB (gp 145) was studied on acetone-fixed cryosections (10 μm) of 8-week-old Balb/c mice internal organs using rabbit antisera (Transduction Laboratories, Lexington, KY) and TRITC-conjugated goat anti-rabbit IgG as the secondary antibody, as described before.[40,41] Sections were counterstained by Hoechst 33342 (Sigma, St. Louis, MO; 10 mg/ml in TBS, 30 minutes) for identification of cell nuclei as described.[42] For detection of p75^{NTR} immunoreactivity, APAAP-staining was performed as described elsewhere,[43] using a monoclonal rat anti-mouse p75^{NTR} primary antibody (Chemicon, Temecula, CA). Sections were counterstained with haemalaun. Incubation of internal organ cryosections without primary antibody was used as a negative control in both experiments. Slides were studied using a fluorescence Zeiss Axioscope microscope and photodocumented using a digital image analysis system (ISIS Metasystem, Altlussheim, Germany).

Specificity Control

The heart of BDNF-overexpressing mice (FVB/N, alpha-myosin heavy-chain promoter[36]) and BDNF−/− knockout mice served as a positive and negative control for ELISA and ISH. Transgenic heart lysates showed 20-fold higher concentrations of BDNF (583.3 ± 151.8 ng BDNF/g total protein) than FVB/N wild-type heart lysates (30.5 ± 3.7 ng/g), whereas BDNF was not detectable in heart lysates of BDNF knockouts. ISH revealed a strong and ubiquitous BDNF mRNA staining in cardiomyocytes of BDNF overexpressors. Wild-type hearts displayed distinct BDNF mRNA-positive cardiomyocytes, BDNF−/− hearts were completely BDNF mRNA-negative (not shown). BDNF levels in back skin lysates (telogen) of Balb/c mice ranged at 8.5 ± 2.1 ng/g (Figure 1A), concentrations of BDNF in total brain lysates at 5.9 ± 1.5 ng/g, according to reported data.[38]

Results

Gastrointestinal Tract

In the gastrointestinal tract, the BDNF protein content differed markedly between organs (Figure 1B). High concentrations of BDNF were detected in the colon and duodenum, low concentrations in the ileum. In the liver and pancreas, BDNF levels were comparable to those measured in the total brain (Figure 1A). In contrast, the submandibular gland contained very low concentrations of BDNF. In order to identify the cellular sources of BDNF, mRNA expression was examined with nonradioactive ISH (Table 1). The internal circular muscle layer of the tunica muscularis was BDNF mRNA positive throughout the intestine. In contrast, the outer longitudinal muscle layer remained negative (Figure 2, colon). An exception was the upper esophagus, where distinct muscle fibers of the outer layer appeared positive (Figure 3, esophagus). BDNF expression was detected in epithelia throughout

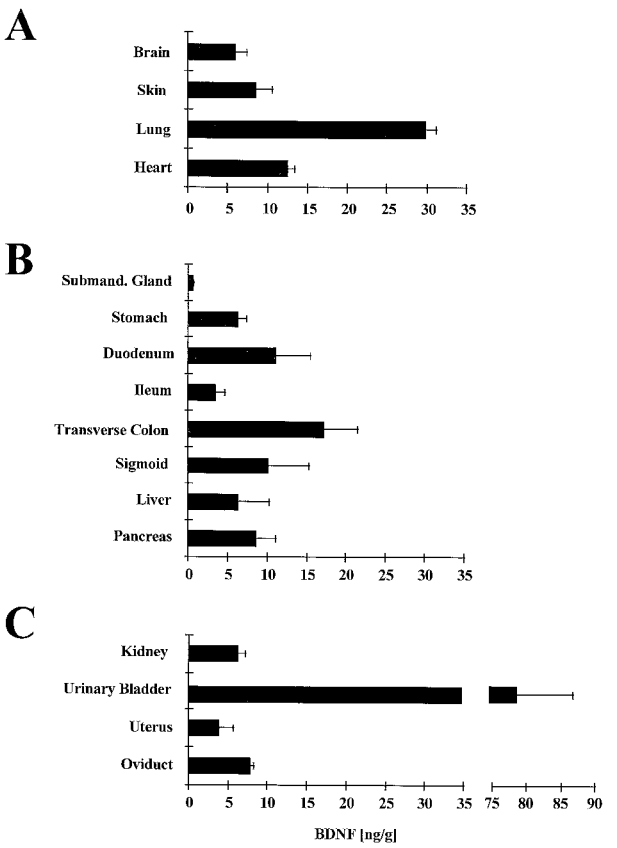

Figure 1. BDNF protein concentrations in internal organ lysates. BDNF protein concentrations in total organ lysates of 8-week-old Balb/c mice were measured by ELISA and calculated as nanograms of BDNF per gram of total protein. Shown are the means with each standard deviation (n = 4). A: BDNF protein in the total brain, back skin and cardiorespiratory system. B: BDNF protein in the gastrointestinal tract. C: BDNF protein in the urogenital tract.

the intestinal tract (Table 1). Intense signals were observed on the bottom of the crypts or of gastric foveolae, respectively. Expression levels appeared to be lower toward the apical cell layers (Figure 2, colon and stomach). Prominent BDNF expression was observed in epithelia of the colon (Figure 2, colon), in contrast, only weak expression was seen in the ileum. Ganglia of the myenteric plexus were BDNF mRNA positive throughout the intestine (arrow in Figure 2, colon). BDNF mRNA was detectable in the peritoneal cell layer of the intestine as on other internal organs (Figure 3, peritoneum). In the liver, hepatocytes were identified as the main sources of BDNF mRNA (Figure 2, liver). Epithelia of exocrine ducts remained unstained in all examined glands (Table 1).

Cardiorespiratory System

A striking pattern of BDNF mRNA-expressing cells was observed in the lung. Respiratory epithelium was strongly BDNF mRNA positive from trachea up to the bronchioli. Airway smooth muscle cells as well as smooth muscle cells of pulmonary vessels (Table 1) were moderately positive (Figure 3, lung). This expression pattern was mirrored in the very high BDNF content measured in lysates taken from lung (Figure 1A). The BDNF protein

Table 1. BDNF Expression and BDNF Receptors in Internal Organs

Organ	Compartment	BDNF mRNA	trkB-IR	$p75^{NTR}$-IR
Submand. gland	acini	+	−	−
	ducts	−	−	−
Sublingual gland	acini and ducts	−	−	−
Esophagus	squamous epithelium	+	−	−
	tunica muscularis	+/−	+/−	−
	myenteric plexus	++	++	++
Stomach	foveolae gastricae	+	−	−
	gastric glands	++	−	−
	tunica muscularis	+/−	−	−
	myenteric plexus	++	++	++
Duodenum	epithelium	+++	−	−
	tunica muscularis	+/−	−	−
	myenteric plexus	++	++	++
Ileum	epithelium	+	−	−
	tunica muscularis	+/−	−	−
	myenteric plexus	++	++	++
Colon	epithelium	+++	−	−
	tunica muscularis	+/−	−	−
	myenteric plexus	++	++	++
Lung	respiratory epithelium	+++	−	−
	airway smooth muscle	++	−	−
	blood vessels	++	−	−
Heart	cardiomyocytes	++/−	−	−
Liver	hepatocytes	++	−	−
	portal triad	−	−	−
Pancreas	exocrine glands	+++	−	−
	exocrine ducts	−	−	−
	islet cells	+	−	−
Kidney	tubules	+++	−	−
	thin segments	+	−	−
	glomerula	−	−	−
Oviduct	columnar epithelium	++	−	−
	lamina propria	+	−	+++
	tunica muscularis	−	−	++
Uterus	columnar epithelium	++	−	−
	lamina propria	+	−	+++
	myometrium	−	−	++
Portio vaginalis	squamous epithelium	−	++	−
Bladder	urothelium	+++	−	−
	tunica muscularis	−	−	−

BDNF mRNA was detected in internal organ cryosections of 8-week-old Balb/c mice by ISH, trkB-IR, and $p75^{NTR}$-IR by immunohistochemistry (see Material & Methods). The intensity of staining is expressed in arbitrary units. −, no staining; +, light staining; ++, moderate staining; +++, strong staining; +/−, stained and unstained portions in the same compartment. trkB-IR, trkB immunoreactivity; $p75^{NTR}$-IR, $p75^{NTR}$ immunoreactivity.

contents found in the heart were significantly lower than those found in the lung. ISH showed that only a few cardiomyocytes were BDNF mRNA-positive (Table 1).

Urogenital Tract

BDNF protein levels in the kidney were comparable to those found in the brain. In contrast, lysates taken from the urinary bladder revealed a much higher concentration of BDNF (Figure 1C). BDNF protein was also detectable in the urine (24.98 ± 14.78 pg/ml), though serum levels were below the detection limit (<4 pg/ml). BDNF protein levels in the oviduct were significantly higher than in the uterus (Figure 1C). For the identification of cellular BDNF expression, BDNF mRNA was detected by ISH. Proximal and distal tubules of the kidney displayed strong BDNF expression. No expression was observed in glomerula (Figure 2, kidney). Renal vascular smooth muscle cells appeared to be BDNF mRNA negative (not shown). Urothelia of the urinary bladder revealed very strong BDNF expression. In contrast, the tunica muscularis was BDNF mRNA-negative (Figure 3, bladder). BDNF mRNA was found in epithelia of the uterus and oviduct. There was also a light expression in adjacent cells of the lamina propria (Figure 3, tuba uterina). Interestingly, BDNF mRNA was not detectable in the squamous epithelium of the portio vaginalis uteri (Figure 3, cervix uteri).

trkB and $p75^{NTR}$ Immunoreactivity (IR)

The strong BDNF expression in visceral epithelia raised the question whether BDNF could also play an autocrine role for non-neuronal structures in the adult viscera. However, almost all epithelia in the examined internal organs were both trkB-IR and $p75^{NTR}$-IR-negative (Table 1). Arrowheads in Figure 4 (colon) show epithelial structures of the transverse colon, which are negative for trkB-IR and

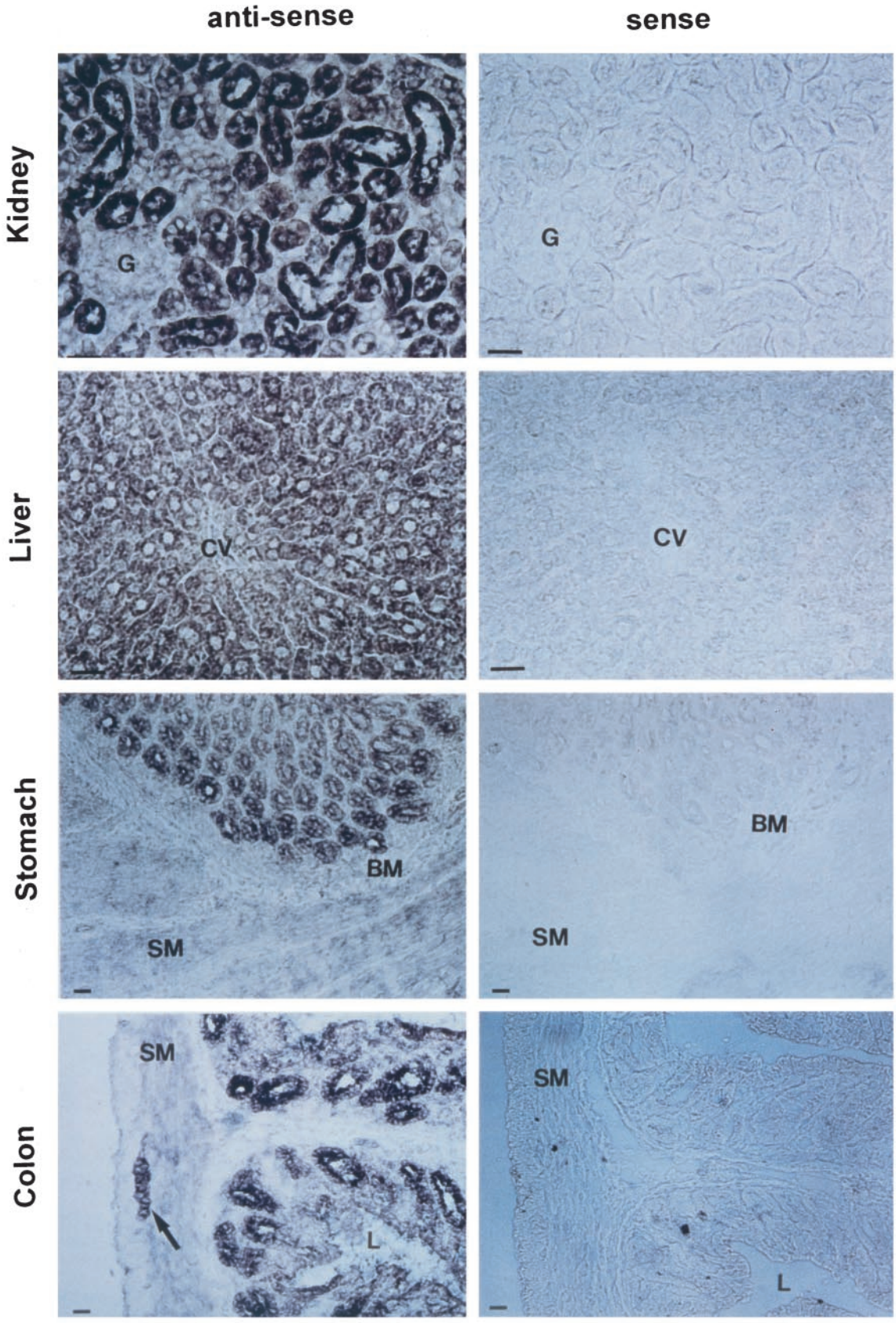

Figure 2. BDNF expression in internal organs. Detection of BDNF mRNA was performed on 10-μm cryosections by ISH. Sequential sections were hybridized with anti-sense or sense riboprobes, respectively. Shown are the cortex of the kidney, a liver lobe with central vein, mucosa and tunica muscularis of the stomach, and sigmoid colon. Note the BDNF mRNA negative glomerula in the kidney. The arrow in the micrograph of the colon shows a BDNF mRNA-positive ganglion of the myenteric plexus. Scale bar, 27 μm. L, lumen; CV, central vein; G, glomerulum; SM, smooth muscle; BM, basal membrane and submucosa of the stomach.

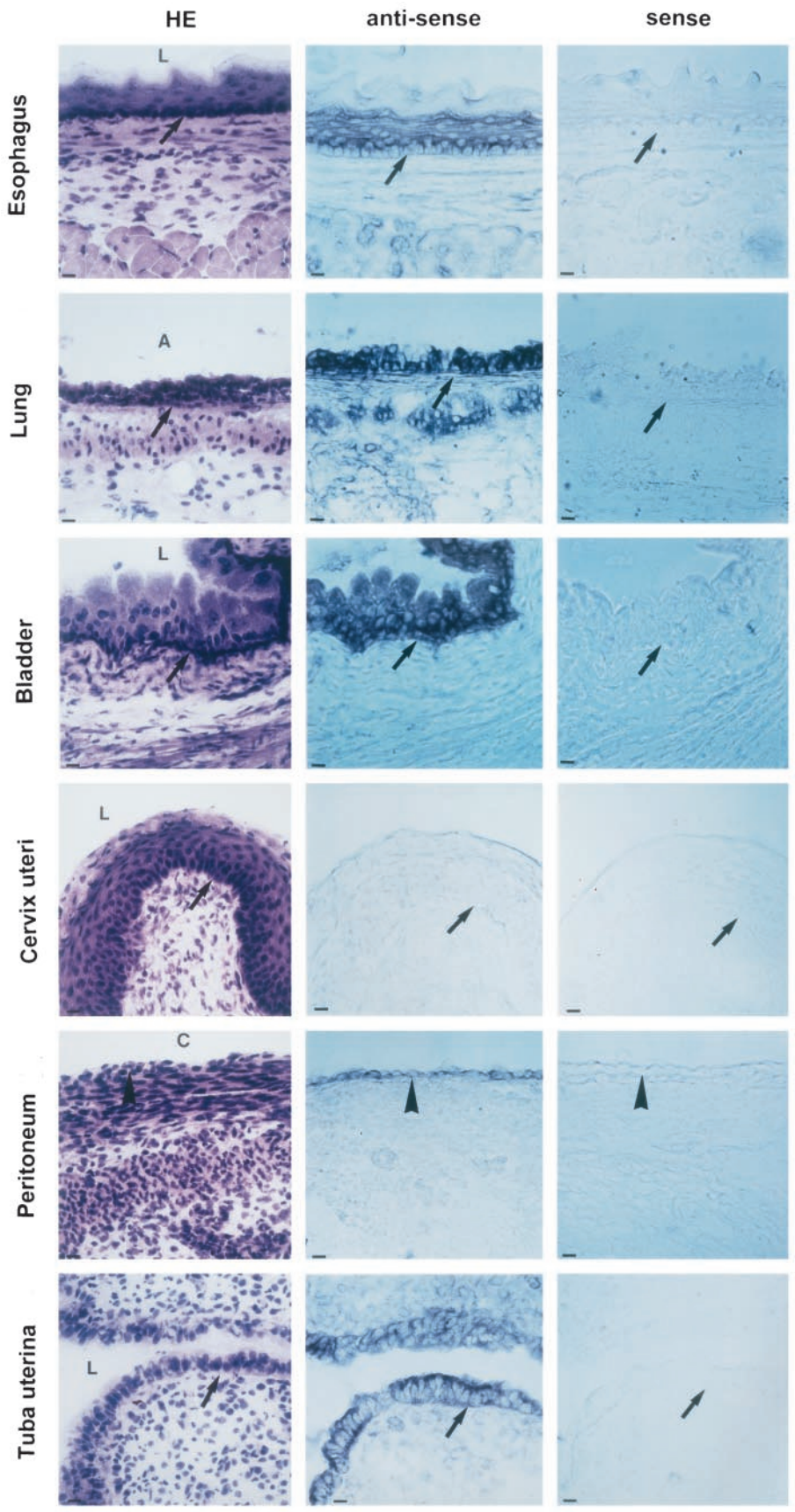

Figure 3. BDNF expression in visceral epithelia. Sequential cryosections were hybridized with anti-sense and sense riboprobes. The first slide of each set was stained with hematoxylineosine (HE). Shown are the mucosa and tunica muscularis of the upper part of esophagus, of a medium-sized bronchus in the lung, of the urinary bladder, the squamous epithelium on the portio vaginalis uteri, mucosa and submucosa of the oviduct, and the peritoneal serosa layer on the myometrium muscle. Arrows indicate the basal membrane of the epithelia. Note that also adjacent stroma cells in the oviduct appear BDNF mRNA-positive. On the negative myometrium background, peritoneal BDNF mRNA signals are easily to identify by blue staining in the peritoneum. Scale bar, 13.5 μm. L, lumen; A, airway; C, peritoneal cavity.

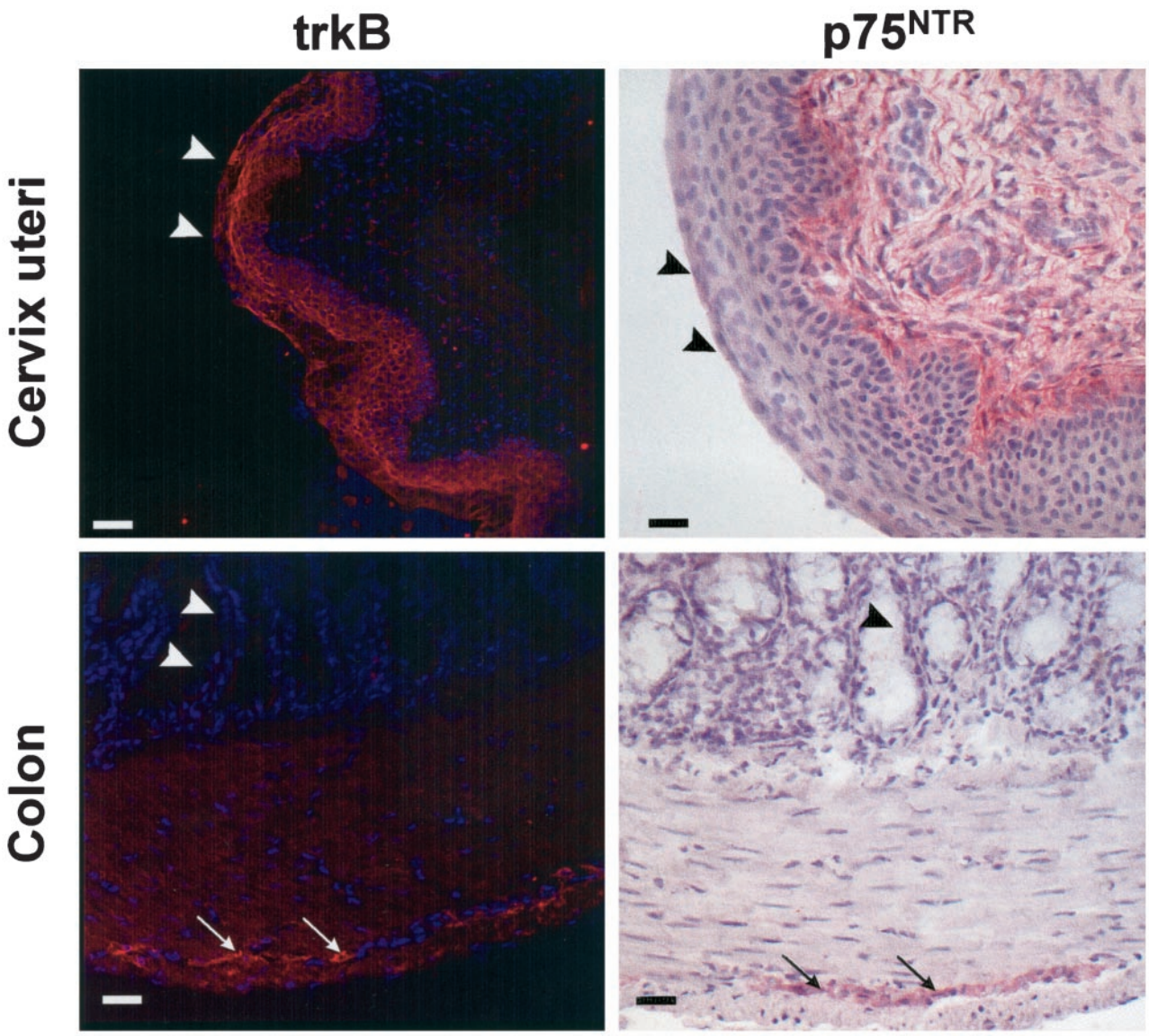

Figure 4. BDNF receptors in the colon and cervix uteri. Immunohistochemistry was performed against the full-length trkB and $p75^{NTR}$ receptors on 10-μm cryosections of Balb/c mice internal organs. Sections were counterstained by Hoechst 33342 (blue fluorescence) for identification of cell nuclei (trkB) or by haemalaun ($p75^{NTR}$). Incubation of inner organ cryosections without primary antibody served as a negative control (not shown). trkB (red fluorescence) or $p75^{NTR}$ staining (red APAAP staining) was evaluated in comparison to this background. Shown are sections of the portio vaginalis uteri and transverse colon. Arrowheads in all figures indicate epithelia, arrows in the micrograph of colon neurons and nerve fibers of the myenteric plexus. Scale bar, 50 μm.

$p75^{NTR}$-IR. An exception was the squamous epithelium of the portio vaginalis uteri, which was trkB-IR-positive (red fluorescence), but $p75^{NTR}$-IR-negative (arrowheads in Figure 4, cervix uteri). Most smooth muscle layers appeared to possess neither trkB nor $p75^{NTR}$ receptors (Figure 4, colon). However, prominent $p75^{NTR}$-IR was detected in the tunica muscularis of the oviduct and in the myometrium (Table 1). Strongest $p75^{NTR}$-IR was revealed in the lamina propria underneath the epithelia of the uterus and oviduct, probably representing connective tissue cells. Figure 4, cervix uteri, shows $p75^{NTR}$-IR (red staining) underneath the squamous epithelium of the portio vaginalis uteri. Scattered trkB-IR was detectable in muscle fibers of the upper esophagus (Table 1). Neurons and nerve fibers of the myenteric plexus showed trkB-IR as well as $p75^{NTR}$-IR (Table 1). White arrows in Figure 4, Colon show trkB-IR (red fluorescence), black arrows $p75^{NTR}$-IR (red staining) on myenteric neurons and nerve fibers of the colon.

Internal Organs of BDNF−/− Knockout Mice

To further analyze the role of BDNF for non-neuronal structures of the viscera, we examined the viscera of mice lacking BDNF. The morphology of internal organs of 2-week-old wild-type ($n = 4$) and BDNF−/− mice ($n = 4$) was examined using HE-stained 2-μm paraffin sections. Throughout the gastrointestinal tract, the intestinal mucosa was present and displayed no gross morphological changes. However, the whole intestine appeared markedly hypotrophic. The ileum and duodenum showed no

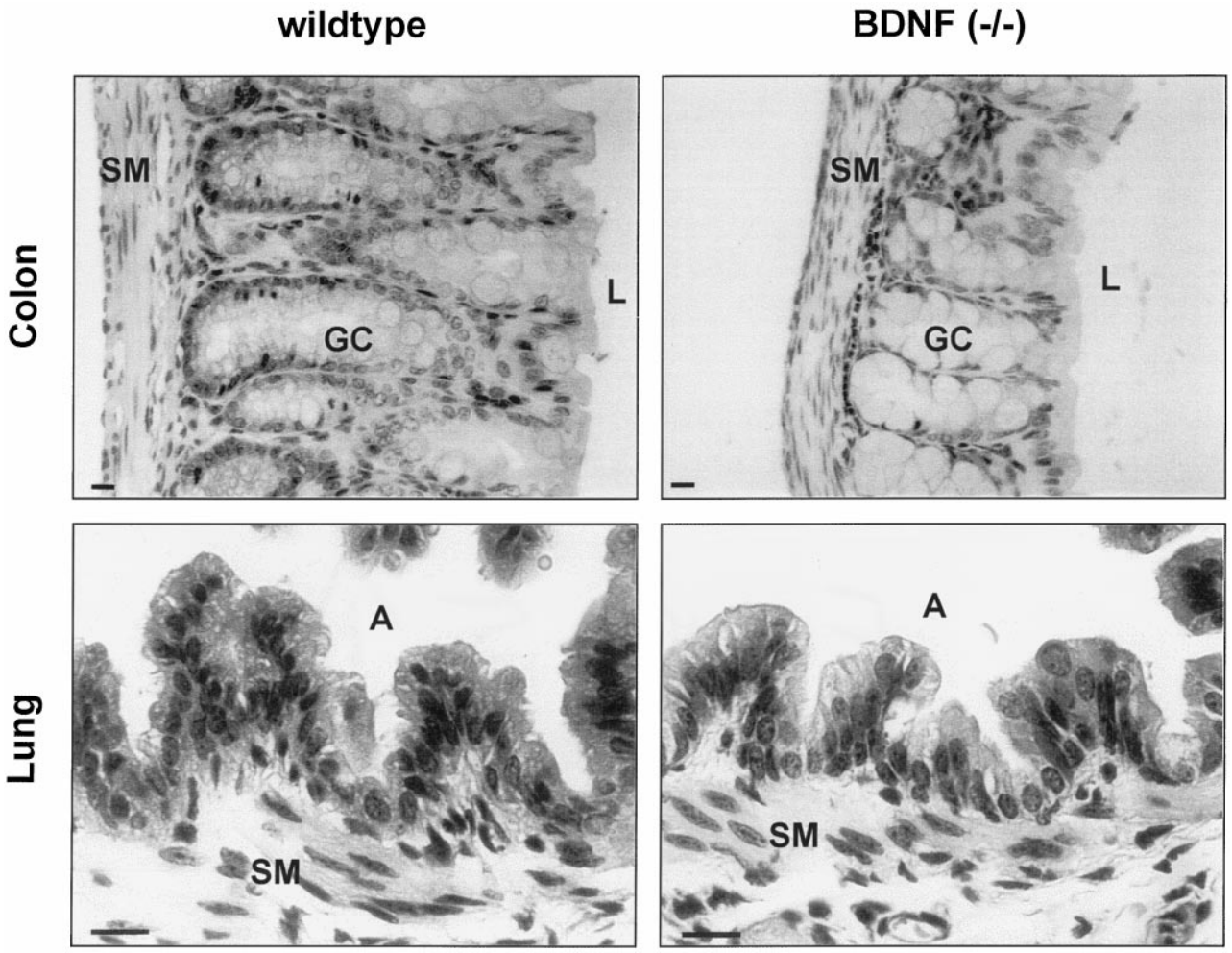

Figure 5. Epithelia of BDNF−/− mice. Two-micron paraffin sections of 2-week-old wild-type and BDNF−/− mice internal organs were HE-stained following standard laboratory procedures. Shown are sections of the transverse colon wall and respiratory epithelium and airway smooth muscle of a main bronchus of wild-type and BDNF−/− mice. Note the mucosal atrophy and enlarged goblet cells (GC) in the BDNF−/− colon. Lungs were indistinguishable between wild-type and BDNF−/− mice; the respiratory epithelium was unaltered in morphology and height. Scale bar, 13.5 μm. L, lumen; A, airway; GC, goblet cells; SM, smooth muscle.

reduction in mucosal thickness and a regular relation of crypts and villi. The colon displayed a mucosal atrophy. Goblet cells of the colon appeared enlarged, probably due to a retention of mucus. Figure 5, colon shows the wall of a wild-type and a BDNF−/− transverse colon. The morphology of pancreatic and hepatic epithelia was unaltered; these organs appeared normal (not shown). Furthermore, the BDNF−/− lung and heart were indistinguishable from wild-type organs. Figure 5, lung, shows the respiratory epithelium and airway smooth muscle of a main bronchus. Epithelial and smooth muscle structures in organs of the urogenital tract (kidney, uterus, oviduct, urinary bladder) appeared unaltered in thickness and morphology as well (not shown).

Discussion

In this study, we describe the expression of the neurotrophin BDNF in visceral organs using ELISA and a sensitive nonradioactive ISH method that allows the identification of the cell types that produce BDNF. The lack of both BDNF receptors on these visceral cells is consistent with previous data from human internal organs.[44] On the other hand, the presence of BDNF receptors on adult PNS neurons innervating the viscera has been shown.[23,45] In addition, we have demonstrated the presence of BDNF receptors on neurons of the adult enteric nervous system (ENS). The uterus showed a unique receptor pattern characterized by an expression of trkB in the squamous epithelium of the portio and $p75^{NTR}$ in smooth muscle and connective tissue cells. Strikingly, these structures appeared BDNF mRNA-negative. Thus, non-neuronal cells of the viscera showed a reciprocal expression pattern of either BDNF or trkB or $p75^{NTR}$. Since BDNF−/− mice exhibit normal epithelial and smooth muscle architecture, but severe deficits in visceral innervation, regulation of neuronal function may be the predominant role of BDNF produced in the viscera.

In the last few years, BDNF mRNA expression has been reported in various gustatory and olfactory sensory epithelia[46–48] and in epithelial cells of the cochlea and vestibulum.[49–52] These sensory epithelia represent inner-

vation targets for neuronal populations, which were reported to be dependent on BDNF. Visceroafferent neurons of internal organs, which are mainly located in the nodose/petrosal ganglion (NPG) and dorsal root ganglia (DRG), were shown to require BDNF during development.[9,10] Recently, it has been shown that during normal development BDNF is transiently expressed at high levels in the targets of arterial baroreceptive and chemoreceptive sensory neurons that have their cell bodies in the NPG.[13] This expression is coordinated with the arrival of sensory axons in these targets.[13] In addition, the onset of trkB expression in neurons of the NPG has been shown to correlate with the onset of BDNF expression in peripheral targets of NPG neurons.[14,27] In the adult animal, retrograde transport of BDNF was recently demonstrated by neurons with their axons in the vagus nerve.[22] A small number of these neurons had their cell bodies in the NPG, but many motoneurons with their cell bodies in the brainstem also retrogradely transported radiolabeled BDNF. Thus, although the presence of trkB-expressing NPG neurons in the adult animal has been disputed,[23,53] motoneurons innervating the viscera appear to be able to utilize endogenous target-derived BDNF.[22] It is also not in dispute that many adult sensory neurons innervating the viscera with their cell bodies in the DRG possess trkB receptors.[23,45] The fact that more sensory neurons in the adult NPG and DRG contain BDNF protein than produce BDNF mRNA further support a role of retrogradely transported BDNF.[23,24,54] The possible function of target-derived BDNF in the adult animal is still relatively obscure, although evidence does exist that this factor can influence the functional properties of mature sensory and motoneurons.[21,55] It may still be the case that BDNF is required for the survival of adult neurons, but, as no conditional knockouts have yet been described, this issue is still open.

Information on BDNF's role in the ENS is very limited. Furthermore, there are conflicting data about trkB receptor expression in human and rat enteric plexuses.[56,57] The finding that BDNF receptors were identified only on neuronal structures of the gut suggest that the observed BDNF expression in epithelial and smooth muscle cells could influence predominantly innervating neurons. Because there are no data available about the role of BDNF in adult myenteric plexus neurons, both survival and nonsurvival functions are conceivable. The examined BDNF−/− mice did not feed properly, as described elsewhere.[11] Though there was some food in the stomach, the intestinal tract appeared nearly empty. Hence, the hypotrophy of the whole intestine in BDNF−/− mice is most likely due to malnutrition. The marked reduction of food intake is probably also the reason that the (completely empty) colon displayed a mucosal atrophy and significant mucus retention in goblet cells.

BDNF levels in the lung and urinary bladder were 5- to 15-fold higher than in total brain lysates and even higher than BDNF levels previously described in the hippocampus.[58] The levels of BDNF in the urinary bladder are, to some extent, consistent with the finding that nearly all adult afferents projecting through the pelvic nerve are trkB-positive.[45] However, the viscera is a relatively sparsely innervated region in comparison to other somatic tissues.[59] It is, therefore, surprising that the amount of BDNF message and protein expressed by certain viscera is so large compared to somatic tissues, eg, the densely innervated skin. BDNF has been described as playing a role in skin innervation.[21,60–62] BDNF protein levels in the skin, however, were significantly lower than in certain inner organs (8 ng/g in the back skin *versus*, e.g., 80 ng/g in the urinary bladder). It thus appears that, at least in adults, visceral BDNF may also play a role in the functional regulation of visceral motor and sensory as well as possibly enteric neurons.[18,63] There is recent evidence demonstrating that BDNF can regulate the capsaicin sensitivity of adult visceral sensory neurons[20] as well as several functional properties of adult motoneurons.[18,19] Furthermore, it has been well established that BDNF plays a nonsurvival role in CNS neurons.[11] Hippocampal neurons especially require BDNF for the expression of synaptic changes associated with long-term potentiation (LTP).[64,65] It is, therefore, conceivable that BDNF, in contrast to developmental stages, could act primarily on functional properties of PNS neurons.

Recent studies demonstrate that inflammatory diseases of the adult viscera are associated with a local up-regulation of BDNF mRNA and protein production. Interestingly, these observations focus on inner organs we found to be the predominant physiological sources of BDNF in adult viscera (lung and urinary bladder). Allergic asthmatic patients respond with a marked increase of BDNF levels during inflammation in the lung after allergen provocation.[17] In a mouse model of allergic bronchial asthma, we demonstrated that this local increase is due at least in part to an up-regulation of BDNF mRNA production in infiltrating immune cells, including macrophages and T cells.[15] The production of BDNF in activated human immune cells has been demonstrated recently.[66] In addition, a strong local up-regulation of BDNF mRNA was demonstrated in the inflamed urinary bladder.[16] It is well established that the closely related nerve growth factor (NGF) contributes to the characteristic neuronal changes in allergic bronchial asthma and in cystitis. In animal models of these diseases, blocking of NGF partly prevented neuronal changes which follow inflammation.[43,67] Therefore, a similar functional role of locally produced BDNF has been suggested in inflammatory conditions. These observations indicate that BDNF could mediate functional neuronal changes in pathological conditions of the viscera, especially of the lung and urinary bladder.

An additional novel finding is the $p75^{NTR}$ expression on smooth muscle cells of the myometrium. A major role of $p75^{NTR}$ has been postulated recently in myogenic differentiation.[68,69] These studies showed that NGF is capable of stimulating myoblast proliferation and differentiation via $p75^{NTR}$. In addition, NGF and $p75^{NTR}$ down-regulation was shown to be essential for the terminal myogenic differentiation. The observed $p75^{NTR}$-IR on the myometrium could, therefore, indicate the plasticity and growth potency of the uterine smooth muscle. Autocrine processes of BDNF seem not to be involved, because the myometrium was completely negative for BDNF mRNA.

Paracrine actions of BDNF, however, have to be considered not only on uterine smooth muscle cells, but also on the squamous epithelium of the portio, because BDNF has been demonstrated recently to promote keratinocyte proliferation via trkB.[70]

In summary, we have shown the extensive cellular BDNF expression in non-neuronal innervation targets of adult murine viscera. Non-neuronal tissues expressing BDNF did not display BDNF receptors and revealed no architectural changes in BDNF−/− mice. The surprisingly high concentrations of BDNF protein in certain internal organs suggest that this protein probably also regulates functional properties of adult PNS neurons innervating the viscera.

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